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Ocular Responses to Ammonia in Broiler Chickens

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SUMMARY. In two trials, 60 male commercial broilers were placed in each of eight environmentally controlled chambers receiving 0, 25, 50, or 75 ppm aerial ammonia from 1 to 28 days. Birds exposed to 25 ppm (lower concentration) ammonia gas developed ocular abnormalities but at a slower rate when compared with birds exposed to 50 and 75 ppm (higher concentrations). Birds exposed to higher concentrations also developed more severe lesions. With little atmospheric ammonia present after 28 days of the grow-out stage, the corneas indicated signs of healing. Lymphocytes and heterophils were seen in the iris at 49 days in ammonia-exposed birds even when ammonia exposure was terminated at 28 days. The lower ammonia concentrations resulted in abnormalities that were slight when compared with those seen at the higher ammonia concentrations. As measured by the incidence of inflammatory infiltrates in the trachea, lung, and air sacs, respiratory tract tissues did not appear to be affected by any tested level of aerial ammonia. The findings in this investigation represent the first report indicating that ammonia-induced uveitis in chickens clears rapidly after exposure to ammonia ceases.

RESUMEN. Respuestas oculares al amoníaco en pollos de engorde.

En dos ensayos, se colocaron 60 machos de engorde en cada una de 8 unidades con ambiente controlado, los cuales recibieron 0, 25, 50 o 75 ppm de amoníaco desde el día de edad hasta los 28 días. Las aves expuestas a 25 ppm (concentración menor) de amoníaco desarrollaron anormalidades oculares, aunque en menor grado, al ser comparadas con las aves expuestas a 50 ppm y 75 ppm (concentración mayor). Las aves expuestas a concentraciones mayores de amoníaco desarrollaron igualmente lesiones más severas. En concentraciones atmosféricas de amoníaco mínimas presentes después de los 28 días de la etapa de crecimiento, las córneas mostraron signos de cicatrización. Se observó la presencia de linfocitos y heterófilos en el iris a los 49 días de edad en aves expuestas a amoníaco aún cuando la exposición al amoníaco había sido suspendida a los 28 días. Las concentraciones menores de amoníaco resultaron en anormalidades leves al ser comparadas con aquellas observadas en aves expuestas a concentraciones mayores de amoníaco. Mediante la evaluación de la incidencia de células inflamatorias infiltradas en la tráquea, pulmón y sacos aéreos, los tejidos del tracto respiratorio no parecieron haber sido afectados por ninguno de los niveles atmosféricos de amoníaco evaluados. Los hallazgos obtenidos en esta investigación representan el primer reporte indicando que la uveítis inducida por amoníaco en pollos de engorde desaparece rápidamente después de terminada la exposición al amoníaco.

Key words: ammonia, broiler, cornea, eye, performance, uveitis

Bacterial activity within poultry litter produces ammonia that is released into the atmosphere of the house. The nitrogenous fractions of fecal material as well as uric acid in poultry droppings are broken down to produce the irritant, colorless ammonia gas (1,6). Ammonia production is influenced by litter conditions such as moisture content, temperature, pH, and ventilation (1,2). Although the ability to breakdown uric acid may be adaptive, not all organisms can complete the breakdown of uric acid into ammonia, groups work collectively to complete the transformation (6). Carr *et al.* (7) ascribed rising ammonia concentrations in the broiler house to energy conservation practices (tightening of the house), to use of limited area brooding, and to reduced ventilation in winter to conserve energy. Litter reuse has exacerbated the problem (17). When used, litter amendments can reduce ammonia levels in the early stages (about the first 2 wk) of the growout (13,17).

Recommended limits on ammonia levels for broiler houses are 25–50 ppm (10,13), and these limits have been promulgated by U.S. regulatory agencies for human exposure. A limit on 8-hr exposure for humans has been set by the National Institute for Occupational Safety and Health (NIOSH) at 25 ppm; the Occupational Safety and Health Administration (OSHA) has established a 50 ppm, 8-hr exposure for humans (24). Ammonia concentration in a commercial broiler house can exceed 100 ppm, especially during the brooding phase if proper ventilation is not accomplished. Ammonia has been reported to be a potential stressor for broilers. Stressors in chickens can reduce their performance, increase their susceptibility to disease, and in some cases, if extreme, can increase their subsequent mortality (9,11).

Numerous studies report the effects of ammonia exposure relative to broiler performance (1,8,12,18). Ocular and respiratory impairment in chickens can result from ammonia exposure and have been reported for a variety of concentrations with different exposure times. Keratoconjunctivitis, an ocular disorder in young chicks, has been attributed to environmental factors in rearing facilities (7). Chickens continuously exposed to 20 ppm of aerial ammonia have been reported to demonstrate signs of discomfort such as rubbing of the eyes, slight lacrimation, loss of appetite, and reduced growth (2). Exposure to aerial ammonia for 72 hr was reported to make chickens more susceptible to Newcastle disease aerosol infection (2). Valentine (22) observed keratoconjunctivitis and tracheitis in broilers exposed to 60–70 ppm ammonia. Charles and Payne (8) evaluated the influence of graded levels of ammonia on the respiration and performance of broilers and pullets. They reported reduced appetite and growth at 78 and 100 ppm compared with birds in ammonia-free environments. Also, respiratory rate was reduced between 7 and 24%.

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Advancements in management technology and housing have occurred with industry growth so that a single farmer routinely cares for 100,000 or more broilers. In addition, genetic enhancements have made the modern commercial broiler very different from the typical broiler of 20 yr ago. The objective of this work was to evaluate the effects of low-level ammonia exposure over time on the eyes of broilers from modern genetic stock while they were reared in simulated, modern management conditions.

MATERIALS AND METHODS

Design and bird management. In two trials, a completely randomized design was used, and broilers were exposed to 0, 25, 50, or 75 ppm gaseous ammonia. Sixty male commercial broilers (508 Ross × Ross, Aviagen North America, Huntsville, AL) at 1 d of age were placed into each of eight environmentally controlled chambers. Routine vaccination (Marek's disease *in ovo* injection and a combination Newcastle disease and infectious bronchitis vaccine spray) of the chicks had been provided before pickup at the commercial hatchery. Chicks were subsequently vaccinated with infectious bursal disease vaccine at the research farm (*via* water) on day 14. Chicks were provided water and food for *ad libitum* consumption under continuous lighting. Nipple waterers and hanging tube feeders were used. To simulate commercial growout conditions, lighting during the first week of growout was bright (approximately 71.8 lux), and was afterward dimmed to approximately 9.7 lux for the remainder of the study. In each chamber, brood temperature for the first 4 days was 32 C with an 18 C dew point. Temperature was reduced 2 C per week through 4 wk of age, to reach a grow-out temperature of 21 C. Each 14.5-m^3 chamber, equipped with single-pass airflow, was ventilated at approximately 5100 liter/min.

Ammonia addition. For quantitative control of aerial ammonia concentration in the chambers, birds were placed on 10 cm of fresh, kiln-dried pine shavings at the beginning of each trial. Thus, previous manure deposits contributed no ammonia to the study. Earlier experience with pen trials has demonstrated that there is little build up of ammonia from waste material deposition during a flock (unpublished data). When using kiln-dried shavings, the wood material absorbs moisture from fecal deposits making ammonia production insignificant. Although stocking densities, as well as waterer and feeder configurations, were similar to commercial conditions, the impact of deposits on the litter was minimal. Literature suggests that under commercial conditions, ammonia generation is delayed on clean bedding. Ammonia flux measurements on rice hulls by Brewer and Costello (4) showed essentially zero flux in the first 10 days.

Anhydrous ammonia was metered continuously into six of the chambers to maintain two chambers each at 25, 50, or 75 ppm. Glass tube, panel-mount flowmeters were used to control ammonia flow into the chambers. No ammonia (0 ppm) was added to the remaining two chambers to serve as negative controls. Temperature control for three chambers (one each at 25, 50, or 75 ppm) was lost in the first experiment; thus, the first trial produced results for a total of five chambers. The aforementioned treatments were each represented by one replicate group in trial 1, whereas two replicate groups represented them in trial 2. At 28 days, ammonia addition was discontinued; the birds were moved to pen facilities (with new kiln-dried shavings as bedding) where they shared a common atmosphere and were grown to 49 days of age. This mimicked commercial conditions where birds experience greater ventilation rates, and thus lower ammonia concentrations, as they grow older.

Before disturbing the chamber atmosphere each day for animal care, ammonia level was determined using Gastec detector tubes (no. 3L and 3La) in conjunction with a Sensidyne/Gastec pump (kit 800, Nexteq, Tampa, FL). During the 28-day exposure period, the average measured ammonia concentration for each treatment level approximated the designated level, but variability increased as ammonia concentration increased. The average concentration for the 25, 50, and 75 ppm treatments were 25 ± 5 ppm, 43 ± 8 ppm, and 74 ± 15 ppm, respectively.

Eye examination. At the beginning of each trial, 10 birds were randomly selected from each chamber, permanently identified, and were given weekly ocular examinations through the remainder of the study. The ophthalmologist did not know the treatment origin of any bird he examined. Biomicroscopy was performed using a Kowa SL-14 portable slit-lamp. During weekly exams, signs of clinical keratoconjunctivitis and anterior uveitis were recorded. Corneal lesions assessed by biomicroscopy were assigned injury scores similar to Thoft's (21) classification. The numerical scale for grading corneal lesions was 0 = normal cornea; 0.5 = not normal but less than 1; 1 = diffuse corneal edema generally over greater than three quarters of the corneal surface; 2 = 1 + a focal superficial corneal ulcer measuring less than one quarter of the corneal surface; 3 = 1 + a corneal ulcer of half or more of the corneal surface and extending into the anterior chamber; 4 = 3 + deeper extension into the stromal layers; and 5 = corneal perforation. The numerical scale for evaluating the anterior chamber was developed during the first trial. This scale is dependent on the definition of a flare, which is the breakdown of the blood–eye barrier or protein leakage across this barrier into the anterior chamber creating cloudiness. The anterior chamber was assessed as 0 = normal anterior chamber; 0.5 = not normal but less than 1; 1 = flare is visible; 2 = flare is easily visible; 3 = flare is easily visible with neovascularization on the iris surface; and 4 = flare is easily visible with hyphema clearly evident and diffuse iris neovascularization.

Histopathologic examination. At the end of each trial (day 49), four birds from each treatment were randomly selected and euthanized by cervical dislocation for histopathologic assessment. Tissue samples, including trachea, lung, air sac, and the entire head, were placed into 10% buffered formalin. After fixation was complete, the left eye was enucleated from each head and placed in a 5% nitric acid solution for 24 hr to decalcify the scleral ossicles. Following decalcification, a longitudinal section was trimmed from each eye through the center of the cornea and then washed in running water for an additional 24 hr to remove the acid. The eye specimens and trimmed specimens of trachea, lung, and air sac were then processed routinely, embedded in paraffin, sectioned at 5 μ, and stained with hematoxylin and eosin. The pathologist was unaware of bird treatment origin. The iris, ciliary body, and conjunctiva were scored for the presence or absence of lymphocytic or heterophilic infiltrates per section. In addition, the presence or absence of increased cellularity along the rostral surface of the iris was noted. Corneal stroma tissue was evaluated as being normal, having vacuolation, or having corneal fibrosis. Tracheas and lungs were scored for the presence of lymphocytic and heterophilic infiltrates per section. The histologist reported the results for the air sacs as a pooled observation, such that only two observations, one from each trial, were available for analysis of these tissues. Lymphoid infiltrates were scored as follows: 1 = none or rare; 2 = lymphocytic infiltrates but no germinal center formation; 3 = one germinal center seen; and 4 = more than one germinal center seen. Heterophil infiltrates were scored as follows: 1 = none or rare; 2 = small numbers (must use 10× lens to confirm presence); 3 = moderate numbers (easily seen at 4×); and 4 large numbers (confluent infiltrates).

Statistical analysis. Analysis of variance was performed combining data across both trials, where trial was a random block effect, and trial × treatment was a component of error. The 0 ppm treatment was omitted from the analysis for the eye examination scores (Table 1), and comparisons were only made among the 25, 50, and 75 ppm ammonia groups. Also, because of the large range among the data, log transformation of the raw scores was used. Geometric means are presented (Table 1) for the corneal and anterior chamber scores. Ten birds per chamber (i.e., 10 birds/treatment in trial 1, and 20 birds/treatment in trial 2) were evaluated to assess the corneal and anterior chamber damage weekly. The histopathologic eye tissue evaluations (presented as percent of occurrence in Table 2) required arcsine transformation before analysis. For each of the four eye tissue, trachea, and lung samples, the presence or absence of lymphocytic or heterophilic infiltrates was given as a positive or negative score. If the number of samples with a positive score was 3 out of 4 for a particular treatment, the percentage of occurrence was 75%. The mixed procedure model of

SAS (19) was used, and treatment means for corneal lesion score, anterior chamber score, and tissue damage were declared different, based on least significant difference (LSD) comparisons at $P < 0.05$.

RESULTS

Eye examination. At day 7, the 50- and 75-ppm treatments exhibited mean corneal lesion scores means of 0.30 and 0.35 (Table 1), where a score of 0.5 is slightly abnormal and a score of 1 is indicative of diffuse corneal edema over more than 75% of the corneal surface. None of the treatments were significantly different at day 7, and normal corneas were observed for the control treatment throughout the growout (Table 1). From days 14–28, corneal lesions were similar for the 50- and 75-ppm treatments, where some birds had corneal ulceration. At the 25-ppm concentration, no birds exhibited ulceration, and changes were significantly less during this same time period. In the absence of the ammoniated atmosphere during days 28–49, healing occurred as evidenced by the drop in mean corneal lesion scores for the 25-, 50-, and 75-ppm treatments.

Anterior eye chamber scores (presented as geometric means) were hardly perceptible at day 7, where the 75-ppm treatment had the only nonzero mean score at 0.04. From days 14–49, the changes in the anterior chamber demonstrated a trend similar to that seen with corneal lesion scores in that the 0- and 25-ppm treatment groups were comparable as were the 50- and 75-ppm groups. However, at day 14, there were statistically significant differences between 25 ppm and the greater concentrations of 50 and 75 ppm. Mean anterior chamber scores were highest at day 21 and day 28 but were statistically the same among all groups receiving ammonia.

No anterior uveitis was observed in birds from the 25-ppm ammonia chambers. However, clinical keratoconjunctivitis and uveitis were observed in birds exposed to 50 and 75 ppm ammonia for 1 wk. These conditions worsened as duration of exposure and concentration of ammonia increased. Birds exposed to 25 ppm ammonia for 3 wk demonstrated keratitis, which was similar to the keratitis observed in birds exposed to 75 ppm ammonia for only 1 wk.

Histopathologic examination. The main histopathologic changes noted were the presence of lymphocytic and heterophilic infiltrates in the iris stroma, ciliary body, and conjunctiva (Table 2), and cellular infiltrates were rare in controls. Of the tissues examined, only in the iris stroma were statistically significant differences found among the treatments for lymphocytes and heterophils. Infiltration of lymphocytes into the iris stroma was not apparent when the 0- and 25-ppm treatments were compared, but they were present in the 50-ppm treatment, which had the greatest occurrence (93.3%). The 75-ppm treatment was statistically indistinguishable from the 25- or 50-ppm treatments. This may have been because of the greater variability in ammonia concentration for the chambers at the 75-ppm level. The evaluation of the iris stroma heterophilic infiltrates showed a low incidence for 0- and 25-ppm treatments whereas the higher ammonia levels (50 and 75 ppm) showed higher incidences. Lymphocytes and heterophils in the ciliary body, although not statistically significant, demonstrated trends similar to those of the iris stroma. There was a trend toward increased cellularity in the iris rostral surface at all levels of ammonia exposure beyond the control, but differences among means were not statistically significant (Table 2).

Histopathologic data for nodular lymphocytic infiltrates in the iris, ciliary, and conjunctiva tissues and for the corneal stroma were excluded from Table 2 because they were virtually undetected. For the conjunctiva, a certain amount of (nodular) lymphoid tissue is probably normal, and its incidence may be highly variable in a population. The treatment means, which ranged from 14.6% to 93.3%, yielded no statistically significant differences. Also, there were no differences in the presence of corneal stroma as related to ammonia exposure. Vacuolation in the corneal stroma was the predominant observation across all treatments. Only one bird had fibrosis, and that bird came from a control treatment.

Table 1. Clinical corneal lesion and anterior chamber scores of male broilers exposed to graded levels of ammonia.[A]

	Days of age				
Ammonia addition (ppm)	7	14	21	28	49
Corneal Lesion Score[B]					
0 (control)[C]	0.00	0.00	0.00	0.00	0.00
25[D]	0.01	0.03[b]	0.40[b]	0.26[b]	0.00
50[D]	0.30	2.09[a]	2.44[a]	2.35[a]	0.77
75[D]	0.35	1.84[a]	2.28[a]	2.11[a]	0.82
Anterior Chamber Score[E]					
0 (control)[C]	0.00	0.00	0.00	0.00	0.00
25[D]	0.00	0.03[b]	0.16	0.14	0.00
50[D]	0.00	0.74[a]	1.51	1.70	0.63
75[D]	0.04	0.74[a]	1.57	1.40	0.82

[A]Means within a column having different superscripts differ significantly based on LSD comparisons ($P \leq 0.05$) on log transformed values.

[B]Numerical scale for grading corneal lesions: 0 = normal cornea, ½ = not normal but <1, 1 = diffuse corneal edema generally over >¾ of the corneal surface, 2 = 1 + a focal superficial corneal ulcer measuring <¼ of the corneal surface, 3 = 1 + a corneal ulcer of ½ or more of the corneal surface and extending into the anterior stroma, and 4 = 3 + deeper extension into the stromal layers, 5 = corneal perforation.

[C]The control treatment was excluded from the analysis of variance and mean comparisons because the control value was always zero. There were 40 observations per mean for the control group.

[D]There were 30 observations per mean for each of the 25, 50, and 75 ppm treatments.

[E]Numerical scale for grading anterior chamber: 0 = normal anterior chamber, ½ = not normal but <1, 1 = flare is visible, 2 = flare is easily visible, 3 = flare is easily visible with neovascularization on the iris surface, and 4 = flare is easily visible with hyphema clearly evident and diffuse iris neovascularization.

Lymphocytic infiltrates in the trachea, lung, and air sac tissue were not different among treatments (Table 3). Heterophilic infiltrates were rarely observed in the trachea, were only slightly more common in the lungs, and had small-to-moderate numbers in the air sacs (Table 3). Heterophilic infiltrates did not differ among the 0-, 25-, and 75-ppm treatment groups. Why the 50-ppm treatment group demonstrated a significantly lower score may be explained by the fact that the air sac scores were reported as a pooled observation resulting in a low number of replications.

DISCUSSION

Broilers exposed to low levels of ammonia gas (25 ppm) experienced ocular changes, and symptoms were exacerbated at higher concentrations (50 and 75 ppm) during the first 28 days. However, once removed from the ammoniated atmosphere, the eyes quickly improved. These findings are consistent with a companion report showing that growth performance recovered from the negative effects of ammonia exposure once ammonia was removed (12). A recovery in body weight was recognized in the later stages of the growout period when birds experienced lower ammonia concentrations. Ocular changes in the present study at 50 and 75 ppm ammonia concentrations included significant corneal ulcerations at 14 days and 28 days of age, and the anterior chamber exhibited

Table 2. Histological changes noted in the iris, ciliary body, and conjunctival tissue (as percentage of occurrence) of male broilers (at 49 d of age) exposed to graded levels of ammonia (24 h/d for 0 to 28 d of age).[A]

	Iris			Ciliary Body		Conjunctiva	
Ammonia addition (ppm)	Rostral surface[B]	Diffuse lymphocytic infiltrates[C]	Heterophilic infiltrates[D]	Diffuse lymphocytic infiltrates[C]	Heterophilic infiltrates[D]	Diffuse lymphocytic infiltrates[C]	Heterophilic infiltrates[D]
0 (control)[E]	6.7	6.7[b]	0.0[b]	6.7	6.7	93.3	14.6
25[E]	85.4	25.0[b]	6.7[b]	6.7	6.7	93.3	45.4
50[E]	90.8	93.3[a]	62.9[a]	25.0	62.9	100.0	9.1
75[E]	93.3	50.0[ab]	62.9[a]	25.0	37.0	75.0	37.0

[A]Means within a column having different superscripts differ significantly based on LSD comparisons ($P \leq 0.05$) on arcsine transformed values.
[B]Observed increased cells along the rostral surface of the iris, which may have been the result of epithelial/endothelial hyperplasia, lymphocytic infiltrates, or both.
[C]Indicates the presence of lymphocytes in the iris stroma, ciliary body, or conjunctiva but does not include lymphocytes that may be present in a nodular aggregate. There were no observations of nodular aggregates of lymphocytes in the iris stroma or ciliary body. Nodular aggregates observed in the conjunctiva were not different among the ammonia treatments.
[D]Indicates the presence of heterophils in the iris stroma, ciliary body, or conjunctiva.
[E]There were eight observations per mean.

abnormalities for the same time period. This is in contrast to a recent report by Beker et al. (3), evaluating four birds per treatment, which showed no corneal lesions after 3 wk exposure to 0, 30, or 60 ppm ammonia. Keratitis, conjunctivitis, and corneal edema, ulceration, and neovascularization have been described in chickens exposed to atmospheric ammonia (20); however, previous reports of uveitis in chickens being caused by ammonia are not evident in the literature.

Lymphocytes and heterophilic infiltrates in the iris generally followed the same trends observed in the cornea and anterior chamber. Furthermore, the incidence of infiltrates at the lower ammonia concentrations was diminished as compared with the higher ammonia concentrations of 50 and 75 ppm.

Because birds were allowed to recover from the ammonia exposure (the ammonia was discontinued at 28 days of age to mimic greater ventilation rate during growout), the corneal tissue from 49-day-old broilers demonstrated few pathologic changes across the treatments. The low occurrence of histologic changes in the cornea coincided with ophthalmologic evaluations at 49 days. When compared with the day 28 examination, noteworthy restoration was evident in the weekly evaluation at day 49. The predominant histologic lesions observed were confined to the iris and ciliary body. Heterophils and lymphocytes were seen across the anterior iris surface and infiltrating the tissue stroma. The threshold for increased heterophilic infiltrate into the iris and ciliary body was 50 ppm atmospheric ammonia. Lymphocytes were noted to accumulate across the anterior iris surface at 25 ppm ammonia but had limited infiltration in the iris stroma at this concentration. Diffuse lymphocytic infiltration of the iris and ciliary body was observed at ammonia concentrations of 50 and 75 ppm.

Tissue from the tracheas, lungs, and air sacs did not appear to be negatively affected at any of the graded treatment levels of aerial ammonia. This can probably be explained by the high solubility of ammonia in water and its efficient removal by the mucous membranes of the upper respiratory system (14). Although changes in the respiratory tract may not be detected in low irritant concentrations, they may be perceptible on a challenge with an airborne infectious microorganism (2). Bullis *et al.* (5) attributed keratoconjunctivitis outbreaks to environmental conditions, particularly ammonia, because no infectious agents could be determined. Oyetunde *et al.* (15) indicated exposure to dust and ammonia created mild-to-moderate macroscopic and microscopic changes to the trachea, lungs, and air sacs, and birds exposed to *Escherichia coli* without these pollutants were relatively unchanged. In that study, combined pathogen/pollutants caused lesions in the respiratory tract and acute inflammatory response in the lungs characterized by vascular congestion, edema, and heterophil and mononuclear cell infiltration. Others indicate deciliation, goblet cell hypertrophy, and epithelial hyperplasia in the trachea with exposure to elevated levels of ammonia (20). The histologic evaluation in the current study might have been expanded to evaluate these changes as the report of

Table 3. Histologic changes noted in the trachea, lung, and air sac of male broilers (at 49 d of age) exposed to graded levels of ammonia (24 h/d for 0 to 28 d of age).[A]

	Trachea[B]		Lungs[B]		Air sac[C]	
Ammonia addition (ppm)	Lymphocytic infiltrates[D]	Heterophilic infiltrates[E]	Lymphocytic infiltrates[D]	Heterophilic infiltrates[E]	Lymphocytic infiltrates[D]	Heterophilic infiltrates[E]
0 (control)	2.88	1.0	2.63	1.63	3.00	2.00[ab]
25	3.25	1.0	2.63	1.25	2.00	2.00[ab]
50	3.63	1.0	2.63	1.25	3.00	1.00[b]
75	2.82	1.0	2.88	1.38	3.00	2.5[a]

[A]Means within a column lacking a common superscript differ based on LSD comparisons ($P \leq 0.05$).
[B]There were eight observations per mean for trachea and lung tissue.
[C]There were two observations per mean for the air sac.
[D]Lymphoid infiltrates scored as follows: 1 = none or rare; 2 = lymphocytic infiltrates but no germinal center formation; 3 = one germinal center observed; 4 = more than one germinal center observed.
[E]Heterophil infiltrates scored as follows: 1 = none or rare; 2 = small numbers (must use 10× lens to confirm presence); 3 = moderate numbers (easily seen at 4×); 4 = large numbers (confluent infiltrates).

lymphocytic and heterophilic infiltrates is inconclusive when compared with the cited literature. Clinically, the birds in this study showed signs of healing, but a complete return to normal cannot be assumed to occur microscopically. These animals may still be more susceptible to secondary disease challenges.

Under modern growout conditions, where house air exchanges increase with increasing bird age, ammonia levels normally decrease. The current study simulated these conditions and found improved eye health once the aerial ammonia was diminished. The study supports the recommendation for 25 ppm aerial ammonia and the need for ammonia control in the early stages of a flock.

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